AMENDMENT

In the Specification:

Please replace the paragraph located just prior to the section entitled "Background of the Invention", on page 1, added in an amendment on June 13, 2000, with the following amended paragraph:

The present specification incorporates herein by reference, each in its entirety, the sequence information on the Compact Disks (CDs) labeled Substitute Sequence Listing Copy 1 and Substitute Sequence Listing Copy 2. The CDs are formatted on IBM-PC, with operating system compatibility with MS-Windows. The files contained thereon were created using Patent-In v. 2.1. The files on each of the CDs are as follows:

Substitute Copy 1 – ~~Seqlist.txt 29KB~~ "SubSeq" 35KB created ~~June 13, 2002~~ October 16, 2003; and

Substitute Copy 2 – ~~Seqlist.txt 29KB~~ "SubSeq" 35KB created ~~June 13, 2002~~ October 16, 2003.

Please delete the paragraph on page 7, lines 20-26, and replace it with the following paragraph:

(18) The HCV polymerase inhibitor of (17), wherein said inhibitor is a polypeptide represented by the formula (I) or a pharmaceutically acceptable salt ~~salf~~ thereof:

Z^1-Z^2-Z^3-Leu-Z^4-Z^5-Trp-Phe-Z^6 (SEQ ID NO: 11) (I)

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wherein Z^1 and Z^6 each represent a hydrophilic group or an amino acid residue; Z^2 and Z^3 each represent a single bond or an amino acid residue; and Z^4 and Z^5 each represent an amino acid residue.

Please replace the paragraph at page 9, line 29, to page 10, line 1, with the following amended paragraph:

"Amino acid sequence suitable for column purification" means any amino acid sequence that can specifically bind to a carrier used for an affinity column used for purification of the polypeptide. Such a sequence is preferably a sequence which can be easily cleaved after the purification with the affinity column and/or does not prevent crystallization of the polypeptide, and includes, for example, the histidine tag sequence, and the glutathione S-transferase ~~S-trasferase~~ tag sequence. The histidine tag needs four or more histidines, and specifically includes - Gly - Ser - His - His - His - His - His - His (residues 572-579 of SEQ ID NO: 3), - Gly - Ser - His - His - Asp -His - His - His (SEQ ID NO: 25), etc.

Please replace the paragraph at page 10a, lines 21-34, with the following amended paragraph:

Examples of the polypeptide represented by the formula (I) include:

Lys-Asp-Leu-Ser-Gly-Trp-Phe-Lys (SEQ ID NO: 12);

Lys-Lys-Asp-Leu-Ser-Gly-Trp-Phe-Lys (SEQ ID NO: 13);

Lys-Asp-Leu-Ser-Gly-Trp-Phe-Val (SEQ ID NO: 14);

Leu-Asp-Leu-Ser-Gly-Trp-Phe-Lys (SEQ ID NO: 15);

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Leu-Asp-Leu-Ser-Gly-Trp-Phe-Val (SEQ ID NO: 16);

Asp-Leu-Ser-Gly-Trp-Phe-Val (SEQ ID NO: 17);

Asp-Leu-Ser-Gly-Trp-Phe (SEQ ID NO: 18);

Leu-Ser-Gly-Trp-Phe-Val (SEQ ID NO: 19);

Leu-Ser-Gly-Trp-Phe (SEQ ID NO: 20);

Leu-Ser-Gly-Trp-Phe-Lys (SEQ ID NO: 21);

Lys-Leu-Ser-Gly-Trp-Phe (SEQ ID NO: 22);

Leu-Gly-Gly-Trp-Phe (SEQ ID NO: 23);

Leu-Ser-Asp-Trp-Phe (SEQ ID NO: 24); *etc.*

Please replace the paragraph at page 10a, line 35, to page 10b, line 4, with the following amended paragraph:

The polypeptide is preferably

Lys-Asp-Leu-Ser-Gly-Trp-Phe-Lys (SEQ ID NO: 12) and

Leu-Asp-Leu-Ser-Gly-Trp-Phe-Val (SEQ ID NO: 16),

and more preferably

Lys-Asp-Leu-Ser-Gly-Trp-Phe-Lys (SEQ ID NO: 12).

Please delete the paragraph on page 12, lines 34-35, and replace it with the following paragraph:

Figure 3 compares amino acid sequences of HCV polymerase (SEQ ID NO: 26), poliovirus polymerase (SEQ ID NO: 27), and HIV reverse transcriptase (SEQ ID NO: 28).

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Please replace the paragraph at page 20, lines 3-10, with the following amended paragraph:

The DNA fragment comprising the histidine tag (SEQ ID NO: 2) consisting of the amino acid sequence of GSHHHHHH at the C-terminus (residues 572-579 of SEQ ID NO: 3 ~~2~~) of NS5B was prepared by PCR using pDM22 into which cDNA of HCV-BK type virus was introduced, purchased from Research Foundation for Microbial Diseases of Osaka University, as a template, and a set of primers 5BNde1FW (SEQ ID NO: 4) and 5B570HRV (SEQ ID NO: 5). The resulting fragment was inserted into pCR2.1 vector (INVITROGEN), the sequence was confirmed, and about 1.8 kDa fragment was obtained by partial digestion with *Nde1* and *EcoR1*.

Please replace the paragraph at page 20, lines 22-30, with the following amended paragraph:

In the amino acid sequence of the obtained native HCV polymerase, methionine at the N-terminus was missing. The amino acid sequence of the obtained $NS5B_{570}$ was the amino acids 1 - 570 of the amino acid sequence shown in SEQ ID NO: 1, to which the histidine tag was added. In the same manner, $NS5B_{552}$, $NS5B_{544}$, $NS5B_{536}$, $NS5B_{531}$, and $NS5B_{591}$ were obtained using primers 5B552HRV (SEQ ID NO: 6), 5B544HRV (SEQ ID NO: 7), 5B536HRV (SEQ ID NO: 8), 5B531HRV (SEQ ID NO: 9), and 5B591HRV (SEQ ID NO: 10), respectively. The amino acid sequence of the histidine tag in the obtained $NS5B_{544}$ was GSHHDHHH

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(SEQ ID NO: 25).

Please replace the paragraph at page 281, line 34, to page 282, line 6, with the following amended paragraph:

Next, a synthetic peptide was prepared by a conventional method and its HCV polymerase-inhibitory activity was assessed. The synthetic peptide consists of the polypeptide region at positions 546 to 551 of HCV polymerase NS5B to both ends of which a Lys residue is attached and represented by the formula Lys-Asp-Leu-Ser-Gly-Trp-Phe-Lys (SEQ ID NO: 12). The synthetic peptide and $NS5B_{544}$ were preincubated at 25 °C for 30 minutes, and the polymerase activity was measured in the same manner as in Example 5. The synthetic peptide inhibited the polymerase activity 40 to 50% at final concentration of 30 μM.

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REMARKS

I. Status of the Claims

Claims 19-39 are pending in the application. Claims 19-29, 32, and 34-36 have been withdrawn from consideration as being drawn to non-elected subject matter. Claims 30, 31, 33, and 37-39 have been finally rejected.

No claims have been amended with the above amendment. The specification has been amended solely to add sequence identifiers for amino acid sequences containing 4 or more amino acids, as required by the Office, or to amend previous sequence identifiers. The amendment also corrects obvious typographical errors. No new matter has been added.

II. Sequence Listing Compliance

The Office states that this application fails to comply with the sequence listing requirements of 37 C.F.R. §§ 1.821-1.825 "because pages 9-10b, and 20, contain amino acid sequences with sequence lengths that are equal to or greater than 4 amino acid molecules and...do not have SEQ ID Nos cited..." Office Action, page 2.

In response, Applicants submit the above amendment and attached Substitute Sequence Listing, and request amendment of the present specification by replacing the original Sequence Listing with the Substitute Sequence Listing filed herewith.

The undersigned states that the content of the substitute paper copy and the substitute computer readable copy of the Sequence Listing, filed herewith, are the

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same, contain no new matter, and conform with Patent and Trademark Office requirements for the Sequence Listing in accordance with 37 C.F.R. §§1.821-1.825.

III. Enablement Rejection

The Office has maintained the rejection of claims 30, 31, 33, and 37-39 under 35 U.S.C. § 112, first paragraph, asserting that the specification, while being enabling for a crystal structure of HCV polymerase using $NS5B_{570, 544, 536}$ and $_{531}$, does not reasonably provide enablement for all HCV polymerase." Office Action, page 3. To support its position, the Office relies on two articles: *Principles of Protein X-ray Crystallography*, J. Drenth, 2nd printing, page 16 (1995) ("Drenth") and *New Focus* article, Science 298:948-950 (2002) ("*New Focus*"). Applicants traverse this rejection.

Contrary to the position of the Office, the evidence of record does not establish a reasonable basis to question the enablement provided for the claimed invention for reasons of record and as supplemented below.

Drenth

To support its position, the Office alleges that "it is well documented that protein crystallization is in essence a trial-and-error method, and the results are usually unpredictable." Office Action, page 4, citing Drenth, emphasis added.

First, Applicants submit that the entirety of the disclosure in Drenth indicates that the proper experimental conditions that lead to predictable crystallization can be determined in some circumstances. Applicants note that Drenth states that the results are "usually" unpredictable, meaning that they are not always unpredictable.

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Drenth then gives an example of how "a failure in the [author's] air conditioning system, causing an unexpected rise in temperature, led to the growth of perfect crystals of an enzyme, which could not be obtained under more normal circumstances." Drenth, page 16. Applicants submit that Drenth thereby suggests that the author accordingly discovered predictable crystallization conditions for the enzyme, albeit after the experimental temperature was unpredictably altered. Drenth then describes how robots may be used "to perform more experiments in the same time and to determine the optimum crystallization conditions more quickly." *Id.* Again, Applicants point out that Drenth thereby indicates that performing the proper experiments can lead to predictable crystallization conditions, since "optimum cystallization conditions" would presumably include predictable crystallization conditions. Accordingly, Drenth indicates that performing the proper experiments can lead to predictable crystallization conditions.

Second, Applicants point out that Drenth's "trial-and-error method" merely refers to testing a wide variety of experimental parameters to determine as-yet-unidentified crystallization conditions. Applicants submit that Drenth's statement merely suggests that when employing "trial-and-error" methods to discover for the first time a method for crystallizing a protein the results are usually unpredictable. In contrast, Applicants' disclosed methods are not mere "trial-and-error" methods for crystallizing HCV polymerase NS5B because Applicants have experimentally determined and disclose in their specification the experimental parameters that result in predictable crystallization of HCV polymerase NS5B, and derivatives thereof. Accordingly, whether or not Drenth's "trial and error" methods lead to

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"unpredictable" results is not germane to whether Applicants' detailed specification enables the full scope of the claims.

Lastly, nothing in Drenth contradicts the Applicants' assertions that predictable HCV polymerase NS5B crystallization conditions are disclosed in the specification or that undue experimentation is not required to practice the full scope of the claims. Moreover, Drenth fails to provide any evidence indicating that the experimental parameters disclosed in the specification do not result in predictable crystallization of HCV polymerase NS5B crystal structures.

Accordingly, the entirety of Drenth does not provide evidence that Applicants' specification fails to enable the full scope of the claims.

New Focus

The Office also cites the *New Focus* article as allegedly "overwhelming evidence" of the unpredictability of the art of protein crystallization, and to further support its assertion that the specification does not enable the full scope of the claims. Office Action, page 4.

Applicants point out that the *New Focus* report, is a retrospective review of only publicly funded structural genomics projects designed to try to "speed up protein structure determination" (page 948, title subheading), "ramp up quickly, and have each lab solve hundreds of new protein structures per year" (*id.*, col.1), attempt to "automate the research to this degree" (*id.*), "turn out protein structures at high speed" (*id.*, cols. 2-3), and "to see whether large-scale protein mapping is feasible" (*id.*, col 3.) *New Focus* discusses generally the difficulty these projects have had in

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meeting these goals. The present claims do not recite any of these structural genomics project goals, nor do they require that the specification enable such goals. Furthermore, *New Focus* does not address the detailed disclosure of the present application, including whether or not the specification describes the present claimed subject matter in such a way as to enable one skilled in the art to make or use the invention. Indeed, the *New Focus* article suggests that success may "improve rapidly as groups gain experience." Page 949, col. 3. Such "experience" may well derive from the detailed disclosure in the present specification.

As an example of the alleged unpredictability of crystallizing further derivatives of HCV protease NS5B, the Office cites "[s]o far, these projects have targeted more than 18,000 proteins but solved the structures of only about 200." (Page 948, column 3, lines 4-6.) As noted above, the projects reviewed in *New Focus* attempted large-scale crystallization and structural analysis of tens of thousands of different protein targets. The present invention involves crystallization of derivatives of a single protein, HCV polymerase NS5B, including derivatives wherein the NS5B is truncated at a C-terminal residue selected from any amino acid residue ranging from residue 531 (Lys) to residue 570 (Arg). The specification describes crystallization and structural analysis of several different HCV polymerase NS5B crystal structures within that range of $NS5B_{531\text{-}570}$ amino acid sequences, $NS5B_{570}$, $_{544}$, $_{536}$ and $_{531}$.

As another basis of distinction, from amongst the tens of thousands of different protein targets discussed in *New Focus*, the publicly funded projects tend to pick targets from "stretches of DNA encoding genes of completely unknown function,

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hunt down their proteins, [and] study the results...." (Page 949, col. 1.) Variations in the predictability of preparing isolated gene products of unknown function may be due in part to the fact that each individual protein "folds into a complex 3D web" (page 948, col. 3) and results in isolated proteins of unpredictable solubility (page 949, col. 2), thus adding unpredictability to preparing purified protein crystals from the tens of thousands of varied targets identified by the 30 far flung publicly funded research projects.

In contrast, the research efforts of a mere two non-publicly funded companies focusing on specific proteins of known function have shown reasonably high rates of successful crystallization and structure mapping of proteins. (Page 950, inset article entitled "*Big Biology Without the Big Commotion.*") For example, *New Focus* discloses that the companies Syrrx and GenomiX have focused on the families of protein kinases or proteases and have so far mapped about 180 different proteins between them, "about the same number produced by the nearly 30 publicly financed programs worldwide..." (*Id.*) Accordingly, and contrary to the Office's reading of the reference, the total disclosure in *New Focus* suggests that focusing the search for protein crystal structures to the family of proteases results in reasonably high rates of successful crystallization and structure mapping.

Perhaps more akin to these non-publicly funded projects discussed in *New Focus*, the present invention focuses on derivatives of a mere single protein species within the family of known proteases, HCV protease NS5B. Furthermore, the Office already admits that the specification enables several derivatives of that single species. Office Action, page 3. In view of *New Focus* and the fact, admitted by the

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Office, that the specification enables crystallization of several specific NS5B derivatives of the invention, Applicants submit that the specification enables crystallization of all of the HCV protease NS5B crystal structures, within the range of $NS5B_{531-570}$ and derivatives thereof, recited in the claims. Accordingly, the evidence of record supports Applicants' assertions that the specification enables the full scope of the claims.

Finally, nothing in *New Focus* contradicts the Applicants' assertions that the specification discloses predictable HCV polymerase NS5B crystallization conditions or that practicing the full scope of the claims does not require undue experimentation. Moreover, *New Focus* fails to provide any evidence indicating that the experimental parameters disclosed in the specification do not result in predictable crystallization of HCV polymerase NS5B crystal structures. Accordingly, *New Focus* does not provide evidence that Applicants' specification fails to enable the full scope of the claims.

Adachi

In Applicants' response dated May 23, 2003, Applicants submitted Adachi *et al.*, Biochim. Biophys. Acta, 1601:38-48 (2002) ("Adachi") as supplemental evidence that methods of crystallizing NS5B polymerases are predictable in the art of protein crystallization.

In response to Applicants' arguments, the Office states

> Adachi *et al.* further document[s] the unpredictability of the art of crystallization. Adachi *et al.* discloses the proteins were produced in similar conditions and the qualities of the crystals were different (page 44, column 2, § 3.4, lines 4-7).

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Office Action, page 4. Applicants disagree, and submit that Adachi does not provide evidence that the art of crystallizing NS5B polymerase is unpredictable.

Adachi states:

> $NS5B_{531}$, $NS5B_{536}$, $NS5B_{544}$, $NS5B_{552}$-LWFm and $NS5B_{570}$-LWFm produced crystals under similar conditions. The qualities of the crystals were different and this is summarized in Table I. We obtained two kinds of structures, one from $NS5B_{570}$ having low RdRp [RNA-dependent RNA polymerase] activity and another from the other five constructs...having high RdRp activity.

Page 44, column 2, § 3.4, lines 7-11.

Applicants submit that Adachi's different crystal "qualities" are merely referring to sequence-dependent differences in structural characteristics of the various NS5B constructs tested, and are not referring to inconsistent or unpredictable methods of preparing the crystals. Applicants point out that Adachi's crystal structure results, and their different "qualities", provided meaningful and useful crystal structure information because Adachi used the NS5B crystal structure data from all constructs tested to hypothesize a detailed sequence-dependent structural model of NS5B RdRp activity. Pages 46-47, and Figure 7. For example, Adachi states that "[f]rom the analyses of [RdRp] activities and [crystal] structures of the recombinant NS5B proteins, [Adachi] summarized the results...and made a hypothesis to account for the activation of NS5B RdRp by the deletion of the C-terminal region or the elimination of the hydrophobic interaction." Page 46, col. 2.

Adachi's ability to attain meaningful and useful structure information for all the NS5B crystals is clear and particular evidence that Adachi successfully crystallized and analyzed all NS5B constructs tested. Therefore, the evidence suggests that

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Adachi's crystallization method resulted in predictable crystallization of the NS5B constructs. Further, as indicated in Applicants' previous response, Applicants and Adachi used similar methods for crystallizing the NS5B constructs. Accordingly, Adachi supports the contention that Applicants' methods for crystallizing NS5B polymerase are predictable.

Moreover, the Office has not demonstrated that the differences in Adachi's crystal "quality" resulted from unpredictable or inconsistent crystallization methods. The evidence demonstrates that Adachi's meaningful and consistent crystal structure data supports the argument that both Adachi's and Applicants' crystallization methods result in predictable crystallization of NS5B polymerases.

Accordingly, for any of these reasons, the Office has not met its burden of showing that the specification fails to enable the full scope of the claims. Applicants therefore respectfully request reconsideration and withdrawal of the rejection.

IV. Obviousness Rejection

Claims 30, 31, 33, and 37-39 remain rejected under 35 U.S.C. § 103(a) as being obvious over Kim *et al.*, U.S. Patent 6,183,121 ("'Kim") in view of *In re Gulack*, 703 F.2d 1381, 1385, 217 USPQ 401, 404 (fed Cir. 1983) taken with Bressanelli *et al.* Proc. Natl. Acad. Sci. USA 96:13034-13039 (1999) ("Bressanelli"). Office Action, page 6. Applicants traverse this rejection for reasons of record and as supplemented below. Again, Applicants submit that Bressanelli, which discloses crystal structures of HCV polymerase NS5B, is not applicable as prior art against the present application because Bressanelli was published after the claimed priority

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dates of the present application (Japanese patent application nos. JP11-188630, July 02, 1999, and JP11-192488, July 07, 1999.)

The Office states that "priority benefit cannot be granted without certified translations of the documents that are in a foreign language." *Id.* Accordingly, with this response, Applicants submit certified English-language translations of the previously filed certified foreign priority documents.

The combination of Kim and *In re Gulack*, absent Bressanelli, fails to teach or suggest all of the recited limitations of the present claims. There is no evidence of record to indicate that the combination of Kim and *In re Gulack* would have taught or suggested the use of the HCV protease NS5B crystal structures to devise the claimed invention. Further there is no evidence of record indicating that one of ordinary skill in the art would have been motivated to combine Kim and *In re Gulack* and modify them to use HCV protease NS5B crystal structures to devise the claimed invention. Accordingly, there is no evidence of record to indicate that the claims are prima facie obvious

Accordingly, withdrawal of this rejection is respectfully requested.

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CONCLUSION

In view of the amendment and remarks presented, Applicants submit that the claims 30, 31, 33, and 37-39 are in condition for allowance and respectfully request reconsideration of the claims, withdrawal of the rejections, and the timely allowance of the pending claims.

Please grant any extensions of time required to enter this response and charge any additional required fees to our Deposit Account No. 06-0916.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW, GARRETT & DUNNER, L.L.P.

Dated: October 22, 2003

By: _____
Charles D. Niebylski
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